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09/975,374 . 101101

#4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit :  
Examiner :  
Serial No. : 09/975,374  
Filed : October 10, 2001  
Inventors : Michel Lazdunski  
: Gerard Lambeau  
: Emmanuel Valentin  
Title : CLONING AND  
: RECOMBINANT  
: EXPRESSION OF  
: MAMMALIAN GROUP  
: XII SECRETED  
: PHOSPHOLIPASE A<sub>2</sub>



22469

PATENT TRADEMARK OFFICE

Docket No.: 1479-R-00

Confirmation No.: 1246

Dated: January 4, 2002

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**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, DC 20231

Sir:

Prior to action on the merits of the case, kindly amend the above-identified application as follows:

**In the Specification** (Clean copy as amended)

**Please replace paragraph 008 (second paragraph on page 3) with the following:**

[008] This invention relates to a mammalian secreted group XII sPLA<sub>2</sub> containing a potential Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP.

**Please replace paragraph 0010 (last paragraph bridging pages 3 and 4 ) with the following:**

[0010] Fig. 1 represents the alignment of the amino acid sequences of sPLA<sub>2</sub>s. In panel A, the full-length sequence of hGXII (SEQ ID NO: 2) is aligned with the amino acid sequences of mouse (SEQ ID NO: 10), rat (SEQ ID NO: 11), bovine (SEQ ID NO: 12) and

*Xenopus* (SEQ ID NO: 13) GXII sPLA<sub>2</sub>s (sequences were deduced from the alignment of different ESTs and from the BAC clone). For some sPLA<sub>2</sub>s, the XX residues indicate that the sequence is partial. The *Arrowhead* indicates the predicted signal peptide cleavage site (32). The active site region containing catalytic site residues that are found in all sPLA<sub>2</sub>s, and the putative Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP are indicated. The level of identity between the mature protein sequence of hGXII and other GXII sPLA<sub>2</sub>s is shown. Panel B shows alignment of the Ca<sup>2+</sup>-binding and active site regions of hGXII (SEQ ID NO: 18) with a representative member of the four other structural classes of sPLA<sub>2</sub>s (hGIB (SEQ ID NO: 14) for GI/II/V/X sPLA<sub>2</sub>s, hGIII (SEQ ID NO: 15) for GIII sPLA<sub>2</sub>s, Conodipine-M (SEQ ID NO: 16) for GIX sPLA<sub>2</sub>, and Rice II (SEQ ID NO: 17) for GXI sPLA<sub>2</sub>s).

**Please replace the paragraph 0015 (last paragraph bridging pages 5 and 6) with the following:**

[0015]        Thus, the invention concerns a novel mammalian secreted group XII sPLA<sub>2</sub> wherein said enzyme contains a potential Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP. The invention concerns more particularly a mammalian secreted group XII sPLA<sub>2</sub> comprising the sequence of amino acids under SEQ ID NO: 2. More particularly, the mammalian secreted group XII sPLA<sub>2</sub> is a human secreted group XII sPLA<sub>2</sub>.

**Please replace the 0034 (last paragraph bridging pages 14 and 15) with the following:**

[0034]        A blastp search with the amino acid sequence of hGXII sPLA<sub>2</sub> against the protein databases stored at the National Center for Biotechnology reveals matches to a variety of sPLA<sub>2</sub>s from mammals, *C. elegans*, plants and animal venoms, suggesting that this

protein belongs to the sPLA<sub>2</sub> family. The homology however appears to be weak (< 35% identity with blast scores lower than 35) and restricted to a short stretch of less than 60 amino acid residues containing the active site domain and the HD catalytic diad, indicating that the hGXII sPLA<sub>2</sub> is unique among all known sPLA<sub>2</sub>s (Fig. 1B). The histidine of HD is thought to function as a general base to deprotonate a water molecule as it attacks the substrate ester carbonyl carbon, and the β-carboxyl group of the adjacent aspartate coordinates directly to the catalytic Ca<sup>2+</sup> cofactor (6,33). Except for 3 cysteines in the active site consensus sequence (SEQ ID NO: 9) CCXXHDXC which match those of other groups of sPLA<sub>2</sub>s, the location of the other 11 cysteines residues in hGXII is distinct from that of other sPLA<sub>2</sub>s (Fig. 1B). Since the structural arrangement of disulfides has been the main basis for designating the different sPLA<sub>2</sub> group numbers, the naming of the new sPLA<sub>2</sub> as hGXII seems appropriate.

**Please replace paragraph 0035 (the paragraph bridging pages 15 and 16) with the following:**

[0035] The homology between hGXII and all known sPLA<sub>2</sub>s is so low that it is difficult to find the Ca<sup>2+</sup> binding loop, which is usually highly conserved and provides 3 of the 4 amino acid ligands for the catalytic Ca<sup>2+</sup> (34). All mammalian group I, II, V, and X sPLA<sub>2</sub>s contain 19 amino acid residues between the most N-terminal residue that serves as a ligand to the active site Ca<sup>2+</sup> (i.e. His-27 of hGIIA) and the catalytic histidine (i.e. His-47 of hGIIA). In contrast, the corresponding distances for hGIII and plant GXI sPLA<sub>2</sub>s are 25 and 23 residues, respectively, hGXII contains a potential Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP with 23 residues between the N-terminal glycine and the putative catalytic

histidine as shown in Fig. 1. This segment is perfectly conserved among all of the GXII proteins found in gene databases. The x-ray structures of groups I, II, and III sPLA<sub>2</sub>s reveal that the Ca<sup>2+</sup> loop contains the consensus segment X<sub>1</sub>CG<sub>1</sub>X<sub>2</sub>G<sub>2</sub>. The backbone carbonyl oxygens of residues X<sub>1</sub>, G<sub>1</sub>, and G<sub>2</sub> coordinate to Ca<sup>2+</sup>, and the backbone NH of G<sub>1</sub> is proposed to donate a hydrogen bond to the carbonyl oxygen of the enzyme-susceptible substrate ester (33,35). The fact that this residue is glycine in catalytically active sPLA<sub>2</sub>s and that mutating this residue to serine lowers catalytic activity by about 10- to 20-fold (35) argues that steric bulk is poorly tolerated at this position. The putative Ca<sup>2+</sup>-coordinating segment of hGXII shown in Fig. 1B fits the consensus sequence of other sPLA<sub>2</sub>s with the exception that G<sub>2</sub> is a proline in hGXII. The prediction based on examination of the x-ray structures of sPLA<sub>2</sub>s is that the hGXII Ca<sup>2+</sup> binding segment should be functional. It contains G<sub>1</sub>, and the backbone carbonyl of the C-terminal proline can coordinate to Ca<sup>2+</sup> since its three extra methylenes, compared to glycine, are sterically allowed because of the location of this residue on the enzyme's surface away from the substrate binding cavity. Interestingly, sPLA<sub>2</sub> isozymes with relatively low sPLA<sub>2</sub> activity from the venom of the banded krait also contain proline in place of G<sub>2</sub> (36).

**In the Claims** (Clean copy as amended)

- 1) (Amended) A mammalian secreted group XII sPLA<sub>2</sub> containing a potential Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP.



$\begin{pmatrix} x & y \\ z & w \end{pmatrix}$



*[Signature]*

T. Daniel Christenbury  
Reg. No. 31,750

TDC:gj  
(215) 563-1810



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COPY OF PAPERS  
ORIGINALLY FILED**In the Specification** (Marked up version)

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[0015] Thus, the invention concerns a novel mammalian secreted group XII sPLA<sub>2</sub>



wherein said enzyme contains a potential  $\text{Ca}^{2+}$  binding segment (SEQ ID NO: 8) GCGSP. The invention concerns more particularly a mammalian secreted group XII sPLA<sub>2</sub> comprising the sequence of amino acids under ~~SEQ ID NO: 2~~ SEQ ID NO: 2. More particularly, the mammalian secreted group XII sPLA<sub>2</sub> is a human secreted group XII sPLA<sub>2</sub>.

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residue on the enzyme's surface away from the substrate binding cavity. Interestingly, sPLA<sub>2</sub> isozymes with relatively low sPLA<sub>2</sub> activity from the venom of the banded krait also contain proline in place of G<sub>2</sub> (36).



**In the Abstract** (Marked up version)

This invention concerns DNA and peptide sequences encoding a novel mammalian secreted group XII phospholipase A<sub>2</sub> wherein said enzyme contains a potential Ca<sup>2+</sup> binding segment (SEQ ID NO: 8) GCGSP and more particularly, a novel human group XII phospholipase A<sub>2</sub>. The invention also concerns the use of this enzyme in methods for screening various chemical compounds.